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Award Number: W81XWH-04-1-0645

TITLE: Chromatin Regulation of Estrogen-Mediated Transcription in Breast Cancer: Rules for Binding Sites in Nucleosomes and Modified Histones that Enhance ER Binding

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REPORT DATE: July 2005

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
01-07-05	Final	07/01/04-06/30/05

4. TITLE AND SUBTITLE
Chromatin Regulation of Estrogen-Mediated Transcription in Breast Cancer: Rules for Binding Sites in Nucleosomes and Modified Histones that Enhance ER Binding

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-04-1-0645

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Saint Louis University
St. Louis, MO 63104

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT: Abstract on next page.

15. SUBJECT TERMS
Estrogen Receptor Chromatin Nucleosomes HMGB2

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	8	19b. TELEPHONE NUMBER *(include area code)* 301-619-7325

Abstract

Binding of ER to estrogen-regulated promoters has been extensively characterized in assays that utilize naked DNA as a template. In contrast, the factors that allow binding of the ER to the estrogen response element (ERE) within the context of chromatin e.g. within a nucleosome, are largely unknown.

To investigate this process, we have reconstituted nucleosomes that contain an ERE located at different translational positions e,g, center, side or edge. Using gel shift assays, we tested whether ER can bind these nucleosomes. We have also found that the non-histone chromatin protein HMGB2 enhances binding of ER to an ERE located at the center of the nucleosome. We determine whether HMGB2 facilitates binding of ER to ERE located at other positions.

The results of these studies indicate: 1.) ER binding to the nucleosome is dependent on the location of the ERE. Binding at the edge is better than at the center or side. 2.) Binding of ER is enhanced by HMGB2 regardless of the position of the ERE and at each position, HMGB2-ER-nucleosome complexes can be detected. 3.) Binding of recombinant ER to ERE sites within the nucleosome was greatly reduced compared to ER obtained from nuclear extracts. This suggests that additional proteins contribute to the ability of ER to bind nucleosomes.

Table of Contents

A. INTRODUCTION

Inappropriate regulation of transcription by the estrogen receptor (ER) has been implicated in greater than 70% of the cases of breast cancer developed each year. Although binding of steroid receptors to DNA is recognized as necessary for induction of most biological responses to steroid hormones, it is generally accepted that chromosomal protein interaction is also necessary. Thus, the initial stage of gene activation is linked to the interaction of steroid receptors with nucleosomes. The nucleosome core particle consists of a histone octamer (two H2A, H2B dimers and one H3-H4 tetramer) with a 146-164 bp DNA wrapped approximately two turns around the octamer. The core histones themselves are believed to play a role as signaling molecules in the modulation of gene activity by repressing or activating gene transcription.

This study was initiated to determine the interaction of the estrogen receptor with a DNA response element present within a nucleosome structure. Transcriptional activators, like the estrogen receptor, must contend with the constrained structure of the DNA within a nucleosome. Contacts between the DNA and the histones probably compete for interactions necessary for the binding of ER. One goal of this study was to determine whether the position of the DNA response element (ERE) within the nucleosome affects the ability of ER to bind the nucleosome. A related goal was to determine whether ER binding was enhanced preferentially at one location by the non-chromosomal protein HMGB2.

We previously identified a unique subset of the H2B and H4 histone proteins, termed CP4 H2B and CP4 H4, that greatly enhance the binding of ER to the ERE. This ability is not shared by either bulk H2B or H4 derived from the general histone pool. Protein sequencing confirmed that CP4 H2B and CP4 H4 are bona fide H2B and H4 and not new isoforms. We postulated, therefore, that CP4 H2B and CP4 H4 have specific postranslational modifications. A second goal of this proposal is to understand the roles of the modified histone proteins in ER binding.

B. BODY.

Aim 1. Determine how the positioning of the ERE within a nucleosome affects binding of ER. Studies from other laboratories indicate that the ability of transcription factors to bind nucleosomes can be greatly influenced by the location of the binding site. Therefore, we hypothesized that the location of the ERE within the nucleosome will affect binding of ER. To test this hypothesis, we proposed to generate nucleosomes with the ERE located at different translational positions. This required the construction of a target plasmid which allowed insertion of the ERE at different locations with respect to the nucleosome localization signal (Fig. 1 A). Using this target plasmid, the ERE was inserted at three different positions e.g. the Bgl II, Nhe I, and EcoRI sites (Fig. 1b,c, d). The 164 bp DNA fragment isolated from these plasmids was used to generate nucleosomes as described in Ruh et al. (2003). The ability of ER to bind these nucleosomes was tested in a gel mobility assay (Fig. 2). This study indicated that ER bound the ERE located at the EcoR1 site (edge) with 3-5-fold greater affinity than the ERE sites located at the Bgl II site (center) or Nhe I site (side). These results differ from those obtained in similar studies with the glucocorticoid receptor (GR), which indicated that GR binds much better to sites at the center rather than the side of the nucleosome (2).

We previously found that HMGB2, a highly abundant protein that alters chromatin and DNA structure, enhanced ER binding to an ERE located at the center (Bgl II site). The results of

the current study, together with our previous results, indicate a HMGB2-ER-nucleosome complex forms regardless of the position of the ERE. This is consistent with the hypothesis that HMGB2 alters the DNA binding activity of ER as opposed to altering the structure of the nucleosome to promote binding of ER.

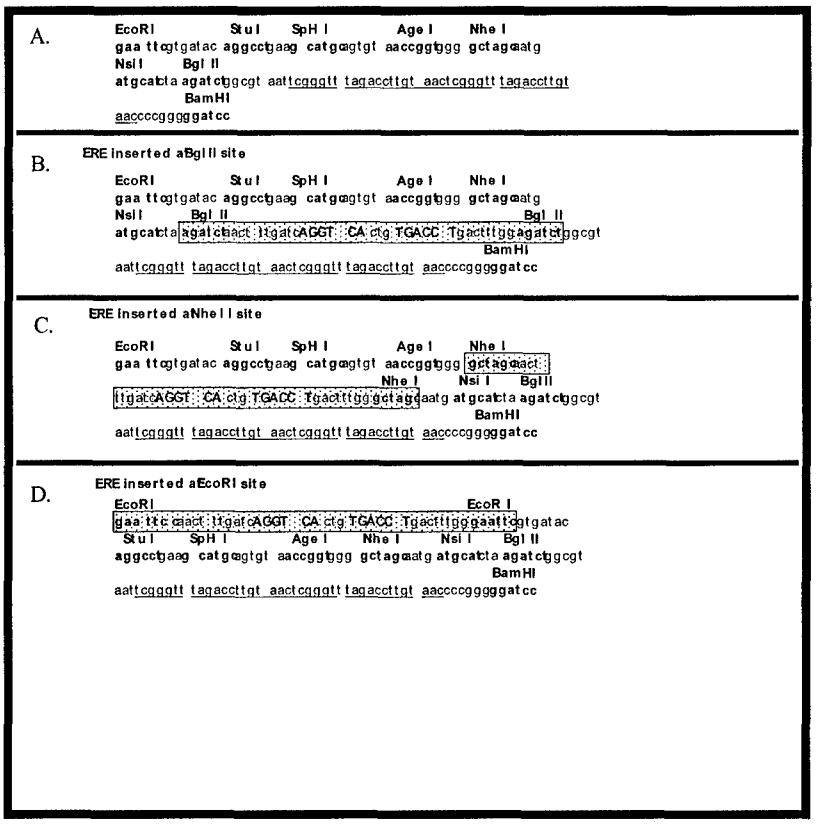

Figure 1. Generation of DNA fragments with the ERE shifted to different translational positions. In (**A**) a multiple cloning site was inserted upstream of the nucleosome localization signal.
In (**B**) the ERE was located at nucleotide 86 at the dyad (center) of the nucleosome by insertion at the Bgl II site.
In (**C**) the ERE was located at nucleotide 66 by insertion at the Nhe I site.
In (**D**) the ERE was located at nucleotide 25 by insertion at the EcoR1 site.

Aim 2. Determine the modifications of histone H2B and H4 that promote interaction of ER with the ERE.

We proposed to identify the posttranslational modifications present in the previously purified CP4 H2B and CP4 H4 histones using mass spectrometry. This approach was not successful. This may have been due to the age of the purified CP4 H2B and CP4 H4 samples and we are currently attempting to generate new samples for completion of these studies. MALDI-MS/MS will also be used in future studies rather than MALDI-TOF.

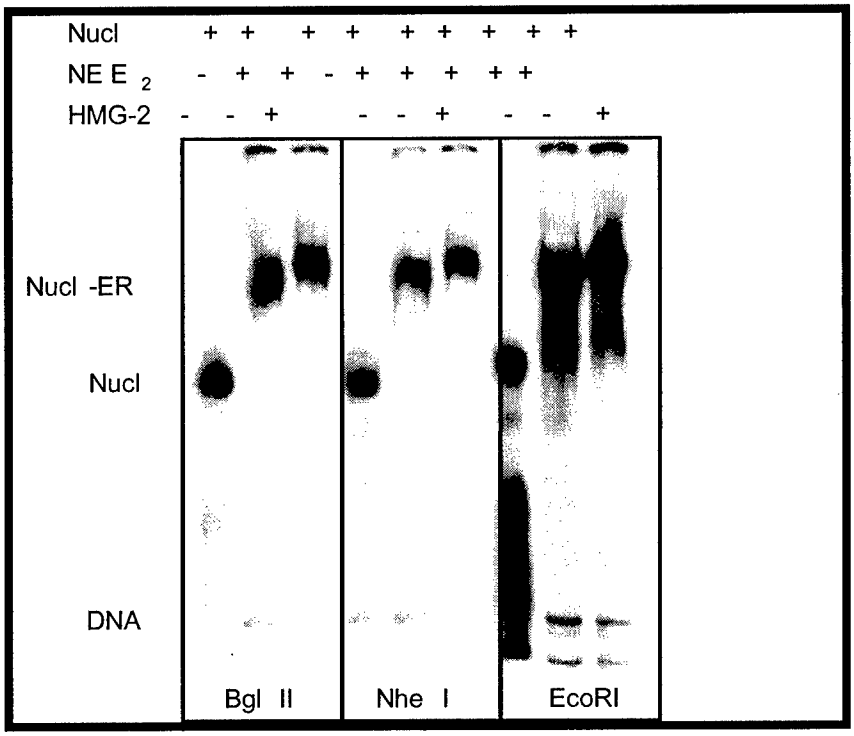

Figure 2. Binding of the estrogen receptor to the ERE site located at different translational positions within the nucleosome. Nucleosomes with the ERE located in the center (Bgl II), at the side, (Nhe I) and at the edge (EcoRI) were incubated with nuclear extracts from MCF7 cells treated with estrogen and where indicated with recombinant HMGB2. Nucleosome (Nucl) and Nucleosome-estrogen receptor complexes (Nucl-ER) were resolved by native polyacrylamide electrophoresis gel.

C. KEY RESEARCH ACCOMPLISHMENTS.

1.) These results establish for the first time that binding of ER to nucleosome is dependent on the location of the ERE. Binding is stronger when the ERE is located at the edge of the nucleosome than at the center. This result could not be predicted from studies using other steroid receptors and indicate further studies to understand the rules that underlie ER binding are warranted.

D. REPORTABLE OUTCOMES.

1.) The results of these findings were presented as a poster at the 2005 Department of Defense Breast Cancer Research Program Meeting (2).

2.) Results of these experiments allowed the PI to apply for an American Heart Grant in 2005, which investigates the role of HMGB2 in estrogen receptor binding to ERE located within nucleosomes. The outcome of this grant is pending.

3.) The results of these experiments have provided additional data supporting the hypothesis that the location of the ERE within a nucleosome affects binding of ER. These results will allow a resubmission of an NIH grant proposal (DK070590-1) focused on examining the effects location of the ERE within nucleosomes has on binding of ER.

E. CONCLUSIONS.

1.) The ability of the ER to bind the nucleosome is dependent on the translational location of the ERE site. ER binding at the edge is better than at the center or side. In contrast, studies with GR indicate it binds better to sites located at the center of the nucleosome compared to other sites. Therefore, the rules, which influence binding of ER and GR to sites within nucleosomes, are different.

2.) Binding of ER is enhanced in the presence of the non-histone protein HMGB2. A HMGB2-ER-nucleosome complex could be detected regardless of the position of the ERE.

3.) The binding of recombinant purified ER to ERE sites within the nucleosome was greatly reduced compared to ER obtained from nuclear extracts. This indicates that additional nuclear proteins are likely to contribute to the ability of ER to bind sites within the nucleosome.

F. LIST OF PERSONNEL

Dr. John Chrivia, Dr. Mary Ruh, Linda Cox, and Natalie Schott contributed to the work in this study.

G. REFERENCES.

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H. APPENDICES

None